Montmorillonite–Cu(II)/Fe(III) oxides magnetic material for removal of cyanobacterial *Microcystis aeruginosa* and its regeneration

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Abstract

In this paper, montmorillonite–Cu(II)/Fe(III) oxides magnetic material was prepared and used for removal of harmful algae from water. The material was prepared by the supporting of Cu(II)/Fe(III) oxides on pillared montmorillonite and was characterized by XRD, zeta potential measurements. The results showed that the whole material is magnetic. The prepared magnetic material was effective for the removal of cyanobacterial *Microcystis aeruginosa* and the loaded particles was chemically regenerated using acetone solution.

Keywords: Montmorillonite; Magnetic oxides; Harmful algae; Water treatment

1. Introduction

Over the last decades, the eutrophication of surface water and algal blooms in lakes and drinking water reservoirs occur worldwide due to the contamination by agricultural activity, domestic wastewater discharge, and industrial effluent. In China, more than 60% of the lakes have been eutrophicated and suffered from harmful algal blooms, where cyanobacterial *Microcystis aeruginosa* (MA) is one of the dominant species [1]. Typically, cyanobacteria

dominate the phytoplankton community and form blooms in eutrophicated water bodies during late summer and autumn [2]. The excessive growth of toxic algae greatly deteriorated the water quality and threatened the drinking water resources. It may cause water quality problems such as taste and odor in water supply. Some algae can penetrate the filter and release toxin, leading to the deterioration of drinking water quality. Otherwise, during the disinfection process of drinking water production, the existence of algae can result in the production of disinfection by-products. In drinking water distribution system, organics released by algae may act as

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assimilable organic carbon (AOC) for growth of biofilm [3–7]. Algal removal by conventional treatment is more difficult than inorganic particle, due to their low specific density, motility, morphological characteristics, and negative surface charge.

In recent years, flocculation with clays has been found to be a promising method for the control of harmful red tide and algal blooms in seas and lakes. Clay is a non-toxic, naturally occurring material and can scavenge particles, including algal cells, from water body and carry them to bottom sediments. Removal efficiencies of 95–99% have been achieved in laboratory cultures using clay, and small-scale field trials near fish farms have also been successful [8–9].

Since clays are effective for the coagulation of red tide organisms, they may also act effectively for the removal of harmful algae from drinking water. Generally, clays with fine particles are more effective. But the fine particles are more difficult to separate from water. Magnetic separation is a promising technology employed in water treatment because of high separation rate using a simple magnetic process. One example of this technology is the use of magnetite particles to accelerate the coagulation of sewage [10]. It seems attractive to combine the removal properties of clay with magnetic properties of magnetic material to produce novel kinds of magnetic material for the removal of algae from water.

In the present research, a magnetic material based on montmorillonite, a commonly used clay, was prepared for the removal of cyanobacterial *M. aeruginosa*. After being mixed with water containing harmful algae, the magnetic material can be separated from water using a simple magnetic separation process. And the regeneration of the recovered magnetic material with algal cells adhering to its surface was also investigated.

2. Materials and experiments

2.1. Culture and measurement of *M. aeruginosa*

Microcystis aeruginosa was cultured according to Pan et al. [1]. For the measurement of residual algal cells concentration, a standard curve was established between the algal biomass concentration and $A_{665\text{nm}}$. Algal biomass was determined gravimetrically as follows: A known volume of algae suspension with different cells concentrations was filtered on a weighed filter, washed with deionized water, dried at 103 °C to constant weight, and weighed. The biomass concentration was determined by the difference of the two weights. Also the same volume of *M. aeruginosa* cells was filtrated onto 0.45 μm cellulose acetate filters and was dissolved using 10 mL 90% acetone solution and then measured for optical density at wavelength of 665 nm ($A_{665\text{nm}}$). The biomass concentration, calibrated against $A_{665\text{nm}}$, was used to monitor the concentration change of *M. aeruginosa* cells during the removal experiments.

2.2. Preparation and characterization of the magnetic material

The magnetic material was prepared by supporting Cu(II)/Fe(III) magnetic oxides on pillared montmorillonite. The preparation procedure for the magnetic material was illustrated in Fig. 1. Na–montmorillonite was used as the starting material. The pillaring solution, aluminium polycation, was prepared by adding 0.5 M NaOH drop by drop to 0.2 M AlCl_3 at 85 °C under vigorous stirring. The mole ratio of NaOH/ AlCl_3 was 2.4. Pillared montmorillonite was synthesized by adding the pillaring solution (aluminium polycation) drop by drop to a 1.5% Na–montmorillonite suspension at room temperature under vigorous stirring. The ratio of aluminum to montmorillonite was 10 mmol g^{-1} . The magnetic dispersion was synthesized by adding NaOH solution

Fig. 1. Preparation pathway for the magnetic material.

to a mixture of 0.533 g $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 2.525 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 0.375 g polyvinyl alcohol (PVA) in 100 mL water till pH = 11.0. The magnetic material was prepared by mixing the magnetic dispersion with pillared montmorillonite suspension at prescribed montmorillonite/ $(\text{CuO} + \text{Fe}_2\text{O}_3)$ mass ratio. The mixture was stirred for 2 h, aged at 95–100 °C for 2 h, then centrifuged and washed with deionized water. The obtained solid was dried, milled (particle size < 0.075 mm), and calcined at 500 °C for 4 h.

Zeta potential of the material was determined by microelectrophoresis using a Malven model Zetasizer 2000 zeta potential analyzer. The zeta potentials of the material suspensions containing 0.02% solid in 0.01 M NaCl were determined at various pH values.

2.3. Removal of algae

Batch experiments were performed in glass bottles. To each bottle, 50 mL algal suspension

of prescribed concentration and magnetic material (1 g L^{-1}) were added. *M. aeruginosa* cells at logarithmic phase were adopted for the experiments. The pH was adjusted using 1 M HCl or 1 M NaOH. The mixture was stirred mechanically at 20 °C with a power basic stirrer for 30 min. Then the magnetic material was separated from the solution by a magnetic process using a permanent magnet made of Nd-Fe-B and the residual concentrations of algae were analyzed.

2.4. Regeneration of the magnetic material

Two kinds of regeneration methods were employed in the experiments: thermal and chemical regeneration methods. For the thermal regeneration experiments, the spent magnetic material was dried, put into a ceramic crucible, and calcined at temperature ranged from 100 to 600 °C for 2 h in an electric muffle furnace under air atmosphere. For the chemical regeneration experiments, acetone solution was used as regeneration solution. The regeneration solution was added to the loaded particles with a regeneration reagent/magnetic material ratio of 50 mL per 1 g. The samples were mechanically stirred at 20 °C for 30 min on a power basic stirrer, then the magnetic material was separated from the medium by the permanent magnet, washed with deionized water and dried.

3. Results and discussion

3.1. Preparation and characterization of the magnetic material

The magnetic material was prepared by supporting magnetic metal oxides on pillared montmorillonite. The supported oxides may have effect on the removal of algae by montmorillonite. The effect of montmorillonite/magnetic modifier ratio for the preparation of the magnetic material, which was expressed in terms of montmorillonite/ $(\text{CuO} + \text{Fe}_2\text{O}_3)$ mass ratio, on the removal efficiency was investigated. The results

Fig. 2. The effect of montmorillonite/magnetic modifier ratio on the removal (*M. aeruginosa* concentration = 0.08 g L⁻¹, pH = 6.8).

were shown in Fig. 2. As can be seen from Fig. 2, with the increase in montmorillonite/(CuO + Fe₂O₃) mass ratio, the removal of algae increased. When the mass ratio equals 1:1, the removal was only 48%. When the ratio increased to 2:1, the removal increased to 92%. After that, the removal did not increase further with the increase in the ratio and a montmorillonite/(CuO + Fe₂O₃) mass ratio of 2:1 was adopted for the preparation of the magnetic material.

Zeta potentials of the magnetic material prepared at different montmorillonite/(CuO + Fe₂O₃) mass ratios at various pH values were measured. The results were shown in Fig. 3. It can be seen that, with the increase in montmorillonite/(CuO + Fe₂O₃) mass ratio, zeta potential decreased, which indicated that, with the increase in montmorillonite/(CuO + Fe₂O₃) mass ratio, material electronegativity decreased. The decrease of material electronegativity facilitated the removal of negatively charged particles from water.

Figure 4 showed the attraction of the magnetic material from water to a permanent Nd-Fe-B magnet. It can be seen that the whole material was magnetic and completely attracted to the magnet. Figure 5 showed the XRD pattern

Fig. 3. The zeta potentials of the magnetic material prepared at various montmorillonite/(CuO + Fe₂O₃) mass ratio.

Fig. 4. Attraction of the magnetic material from water to permanent magnet.

of the magnetic material and it was compared to those of Na-montmorillonite and aluminium-pillared montmorillonite. It can be seen that the $d_{(001)}$ basal spacing of Na-montmorillonite and aluminium-pillared montmorillonite equaled 1.31 nm and 1.98 nm, respectively. This was no $d_{(001)}$ diffraction peak for the magnetic material. These results indicated that the interlayer spacing of the montmorillonite in the magnetic material collapsed. This maybe was due to calcination process during magnetic material preparation.

Fig. 5. XRD pattern of (a) Na-montmorillonite, (b) aluminium-pillared montmorillonite, and (c) the magnetic material.

3.2. Removal of *M. aeruginosa*

3.2.1. Zeta potential of *M. aeruginosa* cells

Before the removal experiments, the zeta potential of *M. aeruginosa* cells was measured and the results were shown in Fig. 6. It can be seen that during the pH range of 3–11, the zeta potential of the cells remained negative. This maybe due to the hydration and ionization of the cell surface. The cell cortexes are comprised of carbohydrates, lipids, proteins, and so on. Because of the excretion and adsorption of cells, there is on the cell surface, a layer of mucilaginous surface adherents, which are organic acids, amino-acids, carbohydrates, and so on,

Fig. 6. Zeta potential of *M. aeruginosa* cells.

containing hydroxyl, carboxyl, amino groups, and so on, which may hydrate or ionize and result in the surface carrying a charge, so that the organism, in a sense, have some colloidal properties even though most are larger than the particles studied by colloid chemists. The colloid properties of the algal cells made them rather difficult to remove from water [11]. SEM photography showed that the *M. aeruginosa* cells are round shaped and are about 2 μm in diameter.

3.2.2. Removal equilibrium

Figure 7 showed the effect of contact time on the removal. The experiments were carried out by stirring magnetic material-algal cell suspension for different time. After magnetic separation, the residual algae were analyzed. As can be seen from Fig. 7, the removal increased with time in the first 20 min and reached equilibrium at 20 min. Removal of *M. aeruginosa* cells by the magnetic material reached 94.8%.

3.2.3. Effect of pH, ion strength, and Ca^{2+} on the removal

The pH value, ion strength, and Ca^{2+} concentration of water may affect surface properties of

Fig. 7. The effect of contact time on the removal (*M. aeruginosa* concentration = 0.053 g L^{-1} , pH = 6.8).

the magnetic material and cells and thus have an effect on the removal. Figure 8 showed the effect of pH on the removal. It indicated that the removal decreased with the increase in pH. Lower pH values facilitated the removal.

Figure 9 showed the effect of ionic strength on the removal. The ionic strength of the cells suspension was adjusted using NaCl. It can be seen that the removal increased with the increase in NaCl concentration. The removal ratio without NaCl is 84.3% and it increases to

Fig. 8. The effect of pH on the removal (*M. aeruginosa* concentration = 0.11 g L⁻¹).

Fig. 9. Effect of ionic strength on the removal (*M. aeruginosa* concentration = 0.067 g L⁻¹, pH = 6.8).

96.1% when NaCl concentration increases to 0.0025 M. The removal increased with the further increase of NaCl concentration and reached 99.0% when NaCl concentration increased to 0.05 M.

Divalent metal cations always exist in lake and reservoir water. In order to assess the potential application of the magnetic material for the removal of algae from natural water, removal capacity was evaluated as a function of Ca²⁺ concentration. The results were shown in Fig. 10. The results indicated that the removal increased with the increase in Ca²⁺ concentration. An increase from 0 to 50 mg L⁻¹ in Ca²⁺ concentration resulted in an increase from 83.5% to 97.4% in removal ratio.

From the above discussion, the removal of algae using the magnetic material seems to be a charge-related process. Zeta potential measurements of the magnetic material and *M. aeruginosa* cells show that in the experimental pH range, the magnetic material particles and *M. aeruginosa* cells were both negatively charged. Electrostatic interaction between magnetic particles and *M. aeruginosa* cells was repulsion. The increase in removal with the decrease in pH and increase in ionic strength maybe was due to the effect of pH, ionic

Fig. 10. Effect of Ca²⁺ concentration on the removal (*M. aeruginosa* concentration = 0.074 g L⁻¹, pH = 6.8).

strength, and cation concentration on surface charge of both the magnetic particles and *M. aeruginosa* cell surfaces.

In water, the surfaces of both the magnetic particle and cells are amphoteric. Their surface potentials are determined by pH value of the suspension. The particles and the cells are less negatively charged at lower pH values. So the decrease in pH reduced the electrostatic repulsion and thus enhanced the removal.

Metal cations can also react with surface groups of the magnetic particles and algal cells and thus affect the surface potential:

The reaction between the divalent metal cation Ca^{2+} and the surface groups decreased the surface potential and thus decreased the electrostatic repulsion between the magnetic particles and algal cells and enhanced the removal.

3.2.4. Effect of cells growth phase

Figure 11 showed the removal of *M. aeruginosa* cells cultured for different period of time. Algal cells at different growth phases (0–9d lag phase, 9–22d logarithmic phase, after 22d senescence phase) were employed. The results showed that the removal efficiency for algal cells at different growth phases fluctuated very little. The removal ratio is during the range of 80–86%.

3.3. Regeneration of the magnetic material

After the removal of algae, the magnetic material was loaded with algal cells and needed to be regenerated. Thermal and chemical regeneration were investigated in this research.

Fig. 11. Effect of cells growth phase on the removal (*M. aeruginosa* concentration = $0.065 \pm 0.05 \text{ g L}^{-1}$, pH = 6.8).

3.3.1. Thermal regeneration

Important aspects of thermal regeneration are the time and temperature required to achieve good regeneration efficiency. The effect of regeneration temperature on removal efficiency of regenerated magnetic material was demonstrated in Fig. 12. The regeneration tests were carried out by calcining the exhausted magnetic material at certain temperatures for 2 h. The results showed that the removal efficiencies of the regenerated magnetic material were during the range of 4–35% and were far below that of the as-prepared magnetic material (94.2%)

Fig. 12. The effect of regeneration temperature on removal efficiency of regenerated magnetic material (*M. aeruginosa* concentration = 0.067 g L^{-1} , pH = 6.8).

at same removal conditions. The experiments further increased the regeneration temperature and found that the enhancing of regeneration temperature did not enhance the removal efficiency of the regenerated magnetic material. The experiments also investigated the effect of calcination time on the removal efficiency of the regenerated magnetic material. The results showed that the removal efficiency of the regenerated magnetic material was not enhanced by optimization of calcination time. In conclusion, calcination cannot achieve good regeneration efficiency.

3.3.2. Chemical regeneration

Acetone solution was selected as regeneration agent in the present work. The effect of acetone concentration on the removal efficiency of the regenerated magnetic material was plotted in Fig. 13. It can be seen that effective regeneration can be achieved using acetone solution as regeneration agent. The removal ratio of the magnetic material reached 81.5% and 82% when the concentration of acetone reached 70% and 90%, while the removal of the as-prepared magnetic material under the same removal conditions was 87%. That is, the chemical regeneration process slightly decreased the removal capacity

and the regenerated material was still effective for the removal of algae.

4. Conclusions

1. Montmorillonite–Cu(II)/Fe(III) oxides magnetic material was successfully prepared by supporting Cu(II)/Fe(III) magnetic oxides on pillared montmorillonite. The montmorillonite/(CuO+Fe₂O₃) mass ratio for the preparation of the magnetic material was determined to be 2:1.
2. The prepared material was wholly magnetic and can be attracted to a permanent magnet.
3. The magnetic material is effective for the removal of cyanobacterial *M. aeruginosa*. The removal increases with the decrease in pH and the increase of ionic strength and Ca²⁺ concentration.
4. The loaded magnetic material particles can be chemically regenerated using acetone solution and the regenerated material is still effective for removal of algae.

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References

- [1] G. Pan, M.M. Zhang, H. Chen, H. Zou, H. Yan. *Environ. Pollut.* 141 (2006) 195–200.
- [2] L.A. Lawton, B.J.P.A. Cornish, A.W.R. Macdonald. *Water Res.* 32 (1998) 633–638.
- [3] J. Ma, W. Liu. *Water Res.* 36 (2002) 871–878.
- [4] J.J. Chen, H.H. Yeh. *Water Res.* 39 (2005) 4420–4428.
- [5] J.D. Plummer, J.K. Edzwald. *Environ. Sci. Technol.* 35 (2001) 3661–3668.

Fig. 13. Effect of acetone concentration on the removal efficiency of the regenerated material (*M. aeruginosa* concentration = 0.086 g L⁻¹, pH = 6.8).

- [6] B.G. Oliver, D.B. Shindler. *Environ. Sci. Technol.* 14 (1980) 1502–1505.
- [7] W. Schmidt, B. Hambsch, H. Petzoldt. *Water Sci. Technol.* 37(2) (1998) 91–96.
- [8] D.M. Anderson. *Nature* 388 (1997) 513–514.
- [9] M.R. Sengco, D.M. Anderson. *J. Eukaryot. Microbiol.* 51 (2004) 169–172.
- [10] N.A. Booker, D. Keir, A. Priestley, C.D. Rithchie, D.L. Sudarmana, M.A. Woods. *Water Sci. Technol.* 123 (1991) 1703–1711.
- [11] Z.M. Yu, J.Z. Zou, X.N. Ma. *Chin. J. Limnol.* 12 (1994) 193–200.